Functions of hyaluronan

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In the middle of the last century, synovial fluid had already been reported to contain a 'mucin' which gave the fluid its viscosity and lubricating properties.[1] In 1939, Meyer *et al*[2] identified the viscous component of this mucin as hyaluronan (hyaluronic acid), a polysaccharide previously isolated from the bovine vitreous body.[3] Subsequent work focused largely on determining the concentration of hyaluronan in, and the viscosity of, pathological synovial fluids[1 4–6] compared with normal fluids. At the same time, other research groups began a physicochemical characterisation of synovial hyaluronan.[7 8] Ever since this pioneering work, the role of hyaluronan in the joint under normal and pathological conditions has continued to arouse interest, especially so after the introduction of intra-articular injections of hyaluronan in the treatment of joint disorders.[9] We have acquired a large amount of knowledge on hyaluronan since the thirties, but do we today know more about its real function in the joints? The structure, physical chemical properties and biology of hyaluronan have been frequently reviewed and the reader is referred to these articles.[10–13]

Structure

Hyaluronan is a linear polysaccharide consisting of alternating units of D-glucuronic acid and *N*-acetyl-D-glucosamine linked by β1-3 and β1-4 linkages.[14] It exhibits polydispersity and the weight-average molecular weight of synovial hyaluronan has usually been reported to be in the order of several millions. The linearity of the hyaluronan structure can be seen in the electron microscope by the Kleinschmidt technique (after binding cytochrome C to the chain). In dilute solution, the polysaccharide behaves as a large solvated coil with a radius of gyration of about 200 nm—that is, it extends over a volume which is a 1000-fold larger than the real volume of the organic material in the chain. The large expansion of the coil is presumably the result of a rigidity in the chain caused by stabilising hydrogen bonds parallel with the chain axis.[15]

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Evidence for an entangled network in semidilute solutions

The large size of the hyaluronan coil leads to entanglement even at concentrations as low as 1 g/l. At higher concentrations the chains form a uniform meshwork throughout the solution. This can be demonstrated by various physicochemical techniques. For example, hyaluronan fractions of different molecular weight sediment at different rates in dilute solution, but at concentrations greater than about 1 g/l they all sediment at the same rate,[16] the rate decreasing with increasing concentration. This is caused by entanglement of the chains at concentrations greater than 1 g/l: a 'plug' is formed which moves slowly through the solution because of the high resistance to flow of solvent through the pores of the plug.[17] This technique has been utilised to calculate the flow resistance of a hyaluronan network.

Viscosity measurements at various concentrations also demonstrate the entanglement. At the point of coil overlap, the concentration dependence of the viscosity changes dramatically and increases exponentially with power of 3.3.[18] The system formed by the network also shows elasticity and shear dependence of the viscosity. The shear dependence disappears in dilute solutions and is thus not due to a deformation of the individual molecules, but to the network.[18 19]

Several investigations indicating specific chain-chain interactions in semidilute hyaluronan solutions have been published. In particular, Welsh *et al*[20] reported that addition of shorter fragments of hyaluronan (about 60 disaccharides) to a high molecular weight hyaluronan solution decreased both the elasticity and the viscosity of the system. They interpreted this as a breakdown of a cohesive network—that is, the short fragments interfered in the interactions between the longer chains. More recently, Scott *et al*[21] used electron microscopy after rotary shadowing of specimens to demonstrate that hyaluronan chains seemed to aggregate into a three dimensional network. They suggested that this was caused by a specific interaction between the hydrophobic surfaces of two chains and the formation of double helical segments[21] or even more complex structures.[22] It has since been shown that addition of oligosaccharides leads to a collapse of the pericellular hyaluronan coat around mesothelial cells;[23] these results were interpreted in terms of competition with the chain-chain interactions in the hyaluronan network.

Our present picture of hyaluronan in synovial fluid is thus that of a network of entangled coils which form a gel-like structure, possibly stabilised by specific but transient chain-chain interactions.

Proposed physiological functions of hyaluronan networks

The rheological properties of hyaluronan solutions showing both viscoelasticity and

shear dependence have been connected with lubricating functions of the polysaccharide in joints and tissues.

WATER HOMEOSTASIS

As mentioned above, hyaluronan exhibits a high resistance against water flow and can thus act in tissues as a barrier against rapid changes in water content. Hyaluronan solutions also exhibit a non-ideal osmotic pressure—that is, the osmotic pressure increases exponentially rather than linearly with increasing concentration of hyaluronan.[24] The latter property gives hyaluronan solutions osmotic buffering capacity, which could be useful in regulating the water content in a tissue.

TRANSPORT

The hyaluronan meshwork acts as a sieve: small molecules move freely in the network, while larger particles are immobilised.[24] This filtering effect was studied in detail and it has been proposed that hyaluronan and other polysaccharides regulate transport of other macromolecules through the extracellular space.

EXCLUSION

The meshwork of hyaluronan chains also excludes other macromolecules from space in the system. The excluded space for a particular molecule increases with increasing molecular size and increasing hyaluronan concentration, and can be quite significant at physiological concentrations. It has been proposed that exclusion regulates partition of proteins between tissue compartments and also influences chemical equilibria of reactions in which macromolecules participate.[24]

Cell biology

The cell biological role of hyaluronan has come into the foreground in the past decade. The discovery of proteins which specifically bind to hyaluronan—hyaladherins[25]—has contributed to this interest. The first hyaladherins to be observed were the aggrecans and link proteins found in cartilage. They bind in large numbers to single hyaluronan molecules, to form gigantic aggregates which are deposited in the cartilage matrix. Subsequently, a number of other proteins with properties of binding to hyaluronan have been reported. In addition, the discovery of the pericellular zone of hyaluronan has added to speculation on the role of hyaluronan-cell interactions in regulating cell activities.

THE PERICELLULAR ZONE

Clarris and Fraser[26] observed as early as 1968 that a hyaluronan containing structure around fibroblasts excluded particles such as erythrocytes from a thick pericellular layer, which otherwise cannot be visualised. The importance of this zone has been documented in developmental processes, as it can prevent cell fusion.[27] The nature of the material in the layer, in addition to hyaluronan, and its attachment to the cell surface have been discussed.

In principle there are two attachment mechanisms proposed: either to hyaluronan synthase operating in the plasma membrane,[28] or to a surface protein recognising the hyaluronan chain.[29] The mesothelial pericellular layer seems to be an example of a 'synthase anchored' coat stabilised by hyaluronan chain-chain interactions.[23] However, it has been shown that cells which carry hyaluronan receptors on their surface can form pericellular layers by addition of exogenous hyaluronan and proteoglycans.[29] This is presumably the mechanism by which proteoglycan aggregates are anchored to chondrocytes.

Various functions of the pericellular layer have been proposed. The layer is part of the complex structure of the extracellular matrix. It can prevent direct contact between cells and protect against attacks from viruses, bacteria, and immune cells. It prevents cells adhering to a substrate, and it has been proposed that, during mitosis, hyaluronan detaches the cell from the substrate so that it can divide. Other cell substrate interactions also could be regulated by the layer.

HYALURONAN BINDING PROTEINS (HYALADHERINS)

The number of proteins reported to recognise hyaluronan specifically is rapidly rising, and the reader is referred to recent reviews.[25 29] Some of these proteins have attracted special attention. When the amino acid sequence of the hyaluronan binding regions of *aggrecan* and *link proteins*[30] had been established, it was found that the lymphocyte homing receptor, CD44, had homologous structures, and it was also shown that certain isoforms of CD44 possessed hyaluronan binding properties.[31] Another cell surface protein, studied by Turley and associates, has been named *RHAMM* (receptor for hyaluronan mediating motility).[32] When hyaluronan binds to this protein, it activates an intracellular tyrosine kinase and induces locomotion of the cell. A third hyaluronan receptor found on liver endothelial cells has been shown to mediate endocytosis of the polysaccharide and has been called the *LEC receptor*.[33] Very recently, McCourt and colleagues have shown that this receptor is identical to the intercellular cell adhesion molecule *ICAM-1*.[34]

The great variety of modes by which hyaluronan can interact with the cell surface indicates that the polymer must have a number of regulatory functions on cellular activities. Hyaluronan has been implicated in mitosis, cell migration, angiogenesis, immune reactions, phagocytosis, and many other processes,[11 13] but research on these interactions must still be regarded as in its infancy.

Hyaluronan turnover in the joint

The general turnover of hyaluronan has been elucidated in the past decade.[11-15 35 36]

Hyaluronan is synthesized in the peripheral tissues. It is partly degraded peripherally and partly carried by lymph to lymph nodes, where another part is endocytosed and degraded. Finally, a minor part is carried to the general circulation, from where it is rapidly removed by the sinusoidal endothelial cells of the liver. The level of circulating hyaluronan is only about 30-40 ng/ml.

Some studies have been specifically directed towards the turnover of hyaluronan in peripheral tissues, including joints.[37]

RATE OF TURNOVER OF HYALURONAN IN JOINTS

Hyaluronan, labelled with tritium in the acetyl moiety, has been injected into knee joints of rabbits.[38] The appearance of tritiated water in blood provided an estimation of the half life of the injected polymer as approximately half a day. The degradation of high molecular weight hyaluronan was only slightly more retarded than that of a preparation of low molecular weight. Similar experiments on sheep suggested a half life of approximately 20 hours.[39] When arthritis had been induced in the joint, the turnover was twice as rapid. These results show a surprisingly rapid turnover for a compound considered by many to have essentially structural and lubricating functions.

SITE OF TURNOVER OF HYALURONAN IN JOINTS

In order to examine at which site synovial hyaluronan is degraded, a polymer substituted with ^{125}iodine-labelled tyramine cellobiose was injected into rabbit joints.[40] Tyramine cellobiose remains within the lysosomes after endocytosis and degradation of the polymer, and it is thus possible to identify the organ in which the polymer has been catabolised. Synovial fluids surrounding joint tissues, regional lymph glands, and liver were collected at different times after deposition of the polymer and analysed for low molecular weight material containing iodine-125. No degradation of hyaluronan appeared to have occurred in the synovial cavity. A large part of the radioactivity was accumulated in the joint tissues: after 24 hours, 30% of the injected dose was recovered here as low molecular weight radioactive material. However, a considerable part of the radioactivity was also found in the liver, suggesting that a significant part of the injected polymer had been carried to the general circulation via the lymph and taken up by the liver. The cells responsible for the catabolism of hyaluronan in the joint capsule have not yet been identified.

Discussion

Do the accumulated data on hyaluronan shed any more light on the function of hyaluronan in the joint? At least they open the door for new hypotheses, which can be tested.

Lubrication. The proposal that the viscoelastic and non-Newtonian behaviour of hyaluronan are important for lubrication should be possible to test with the aid of medium sized hyaluronan oligosaccharides, which would competitively inhibit the interactions which seem to be responsible for the marked rheological behaviour.

Does hyaluronan prevent tissue formation in the cavity? It is quite plausible that the gel-like structure of hyaluronan excludes tissue forming elements from the synovial space. However, it is also possible that, through specific interactions with cells, hyaluronan can regulate cellular events such as angiogenesis, which are required for tissue formation. We should reopen the discussion held many years ago on the role of hyaluronan in acellular organs such as the vitreous body and synovial fluid.

Does hyaluronan have a scavenger function? Why does hyaluronan turn over so rapidly? One possibility could be that hyaluronan has a scavenging function in the synovial cavity. Debris and inflammatory elements which enter the cavity may become stuck in the hyaluronan structure, both mechanically and by specific binding, and should be carried away from the cavity at the same rate as the polysaccharide. Hyaluronan would thus help to keep the joint clean. As hyaluronan is carried into the joint capsule or to the lymph, the debris would presumably be cleared by phagocytes in the lining of the cavity and in lymph nodes.

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